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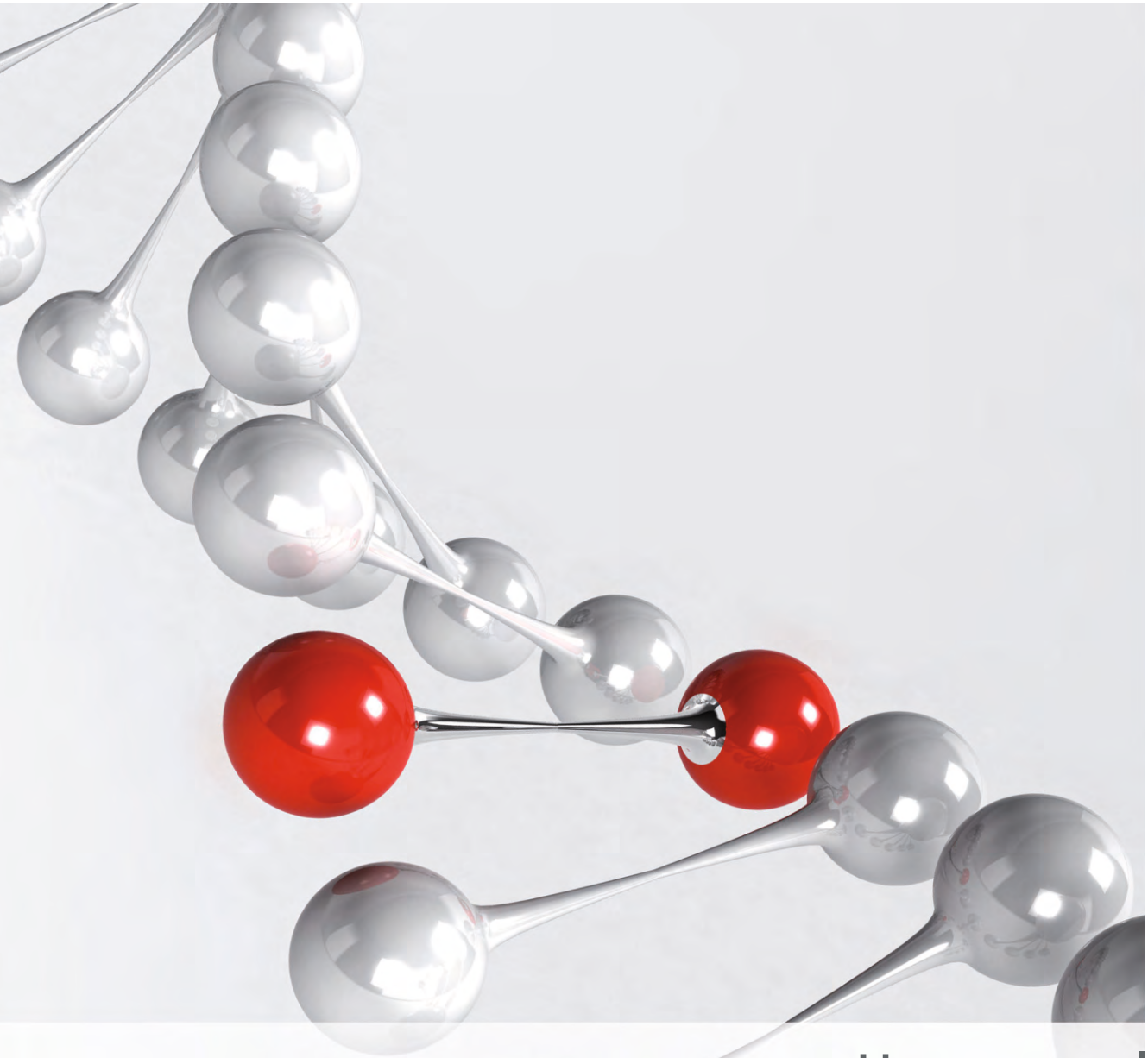

User manual
Invisorb® Spin Plasmid Mini Two

for isolation of pDNA from 0.5 - 2.0 ml bacteria suspension

REF 1010140x00 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for Invisorb® Spin Plasmid Mini *Two*

The **Invisorb® Spin Plasmid Mini *Two*** provides an extremely fast and very convenient tool for an efficient purification of low and high copy plasmid DNA from 0.5 to 2 ml bacteria suspension in comfortable spin format or on a vacuum manifold.

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The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

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The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of Invisorb® Spin Plasmid Mini *Two*

Store all kit components at room temperature!

	10 pDNA extractions	50 pDNA extractions	250 pDNA extractions	500 pDNA extractions
Catalogue No.	1010140900	1010140200	1010140300	1010140400
Solution A	2 x 2 ml	15 ml	70 ml	140 ml
Solution B	2 x 2 ml	15 ml	70 ml	140 ml
Solution C	2 x 2 ml	15 ml	70 ml	140 ml
Wash Solution	10 ml (ready to use)	12 ml (final volume 60 ml)	1 x 40 ml (final volume 1x 200 ml)	2 x 40 ml (final volume 2x 200 ml)
Elution Solution	2 ml	10 ml	30 ml	60 ml
Spin Filter	10	50	5 x 50	10 x 50
2.0 ml Receiver Tubes	10	50	5 x 50	10 x 50
1.5 ml Receiver Tubes	10	50	5 x 50	10 x 50
Manual	1	1	1	1
Initial steps	**Wash Solution** is ready to use!	Add 48 ml 96-100% ethanol to the bottle **Wash Solution**.	Add 160 ml 96-100% ethanol to each bottle **Wash Solution**.	Add 160 ml 96-100% ethanol to each bottle **Wash Solution**.

Reagents and equipment to be supplied by user

- Microcentrifuge
- Measuring cylinder (250 ml)
- Pipet and pipet tips
- Disposable gloves
- Reaction tubes (1.5 ml or 2.0 ml)
- Vortexer
- 96-100% ethanol

Storage

The **Invisorb® Spin Plasmid Mini *Two*** should be stored dry, at room temperature and is stable for at least 12 months under these conditions. Make sure that all components are at room temperature. If there are any precipitates in the reagents provided, dissolve them by careful warming up to 37°C.

Room temperature (RT) is defined as ranging from 15 - 30°C.

Symbols

Manufacturer

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **Invisorb® Spin Plasmid Mini *Two*** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **Invisorb® Spin Plasmid Mini *Two*** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **Invisorb® Spin Plasmid Mini *Two*** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:
from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **Invisorb® Spin Plasmid Mini *Two*** provides a fast, efficient, robust and low cost purification system for plasmid DNA from **0.5 ml to 2 ml** of overnight bacterial cultures or bacterial pellets.
The **Invisorb® Spin Plasmid Mini *Two*** combines the alkaline lysis procedure for the isolation of plasmid DNA with the very efficient binding of plasmid DNA onto a Spin Filter surface.
The isolation protocol as well as all buffers is optimized to provide high yield and purity of the isolated plasmid DNA. The "hands-on time" necessary for the whole procedure is reduced to a minimum.

Plasmid DNA purified by the **Invisorb® Spin Plasmid Mini *Two*** is ready to use for a broad panel of downstream applications.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification.

Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The Kit is neither for isolation and purification of DNA fragments or genomic DNA, nor for isolation and purification of RNA.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

European Community risk and safety phrases are listed below for the components of the **Invisorb®** **Spin Plasmid Mini *Two*** to which they apply are listed below as follows:

Solution C

harmful

H302	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
in USA : 1 – 800 – 535 – 5053

Product characteristic of Invisorb® Spin Plasmid Mini *Two*

Starting material	Yield	Time for preparation	Ratio
0.5 - 2.0 ml bacteria suspension bacterial pellets from max. 2 ml suspension	up to 20 µg	< 15 min	A_{260} : A_{280} 1.8 – 2.1

The **Invisorb® Spin Plasmid Mini *Two*** allows for the purification of up to 20 µg plasmid DNA. The kit provides a fast, simple, and cost-effective plasmid mini preparation method for routine molecular biology laboratory application.

Harvested bacteria from an overnight culture are resuspended and processed by the alkaline lysis in the presence of RNAse, followed by a neutralization step, an adjustment of the binding conditions and after the clarification of the lysate from precipitated proteins and cell unlysed debris the pDNA binds to the membrane of the spin filter. All impurities are efficiently removed by washing and the ultra pure pDNA is eluted in a low-salt buffer.
The kit provides reproducible recovery rates of highly purified pDNA. The "hands-on time" necessary for the whole procedure is reduced to a minimum.

Due to the high purity, the isolated pDNA is ready to use in downstream applications, such as:

- PCR*
- Enzyme Digestion
- Sequencing
- Cloning
- *In-vitro* translation

To purify plasmid DNA in convenient 96 well format, STRATEC Molecular offers the **Invisorb® Plasmid HTS 96 Kit** for use in centrifuge.

*)The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **Invisorb® Spin Plasmid Mini *Two*** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Principle and procedure

The **Invisorb® Spin Plasmid Mini *Two*** procedure is comprised of following steps:

1. growth of bacterial culture *****
2. alkaline lysis of cells, precipitation of proteins and genomic DNA
3. adsorption of plasmid DNA to the membrane of the spin column
4. removal of contaminants and elimination of ethanol
5. elution of plasmid DNA

All steps are performed without use of phenol, chloroform, CsCl, ethidium bromide, and without alcohol precipitation.

This manual contains three protocols.

Lysis
After harvesting and resuspending using Solution A, the bacterial cells are lysed in the presence of Solution B. Solution A contains RNase which digests the RNA during lysis. SDS solubilizes the proteins and phospholipids of the cell membrane. This leads to the lysis of the cells and the release of cell contents. During the subsequent neutralization of the lysate, chromosomal and pDNA as well as proteins will be denatured. The optimal lysis time allows maximum release of the pDNA without release of chromosomal DNA.

Attention: *Long exposure of the pDNA to alkaline conditions may cause the plasmid to become irreversibly denaturated. This denaturated form of pDNA runs faster on agarose gel and is resistant to restriction enzyme digestion.*

During this step the lysate is neutralized and adjusted to binding conditions in one step by the addition of **Solution C**. The proteins are denatured; chromosomal DNA, cellular debris, and SDS are precipitated, while the smaller plasmid DNA renatures correctly and stays in the solution. It is important that the solution is mixed thoroughly and gently to ensure complete precipitation.

Attention: *To prevent contamination of plasmid DNA with chromosomal DNA, vigorous shaking and vortexing must be avoided during lysis. Separation of plasmid from chromosomal DNA is based on coprecipitation of the cell wall-bound chromosomal DNA with insoluble complexes containing salt, detergent, and protein. Plasmid DNA remains in the clear supernatant. Vigorous treatment during the lysis procedure will shear the bacterial chromosome, leaving free chromosomal DNA fragments in the supernatant. Since chromosomal fragments are chemically indistinguishable from plasmid DNA under the conditions used, the two species will not be separated on Spin Filter membrane and will elute under the same low-salt conditions. Mixing during the lysis procedure must therefore be carried out by slow, gentle inversion of the tube.*

Binding plasmid DNA
After cleaning of the supernatant each lysate is applied to an Invisorb® Spin column and plasmid DNA is adsorbed onto membrane while the digested RNA, cellular proteins, and metabolites remain in the lysate and are drawn through by centrifugal force.

Removing residual contaminants
Contaminations like endonucleases or others are efficiently washed away using **Wash Solution**, while the plasmid DNA remains bound to the membrane.

Attention: *This washing step is essential when large cultured volumes are used or when working with endA$^+$ strains such as the JM series, HB101 and its derivates, or any wild-type strain, to ensure that plasmid DNA is not degraded.*

Elution of plasmid DNA
Plasmid DNA is eluted from the column using 50 - 100 µl Elution Solution.
Eluting twice each with 100 µl results in a slightly higher yield of DNA. By the use of small elution volumes DNA concentration can be raised.
Elution volumes should not fall below 50 µl, otherwise the yield will be reduced.
The eluted DNA is ready to use in different downstream applications.

***Note**:* *The **Elution Solution** contains **EDTA**.*
Elution efficiency depends on pH. The maximum elution efficiency is achieved between pH 7.0 – 8.5. When using water for elution, make sure that the pH value is within this range. Store pDNA frozen at –20°C when eluted with water since pDNA may degrade in the absence of a buffering agent.

DNA yield
Plasmid yield with **Invisorb® Spin Plasmid Mini *Two*** procedure varies depending on plasmid copy number per cell (see page 19), the individual insert in a plasmid, factors that affect growth of the bacterial culture (see page 19), the elution volume, and the elution incubation time. A 2.0 ml overnight culture can yield 5 – 20 µg of plasmid DNA. To obtain the optimum combination of DNA quality, yield, and concentration, it is recommend to use an LB-medium for growth of cultures, using elution volume of 50 µl, and performing incubation (1 – 3 min) after addition of the Elution Solution.

***) Growth of bacterial cultures**

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic. The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotic, and type of culture medium.

Plasmid copy number
Plasmids vary widely in their copy number per cell, depending on their origin of replication (e.g., pMB1, ColE1, or pSC101) which determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and cosmids are generally present in lower copy numbers. Very large plasmids and cosmids are often maintained at very low copy numbers per cell.

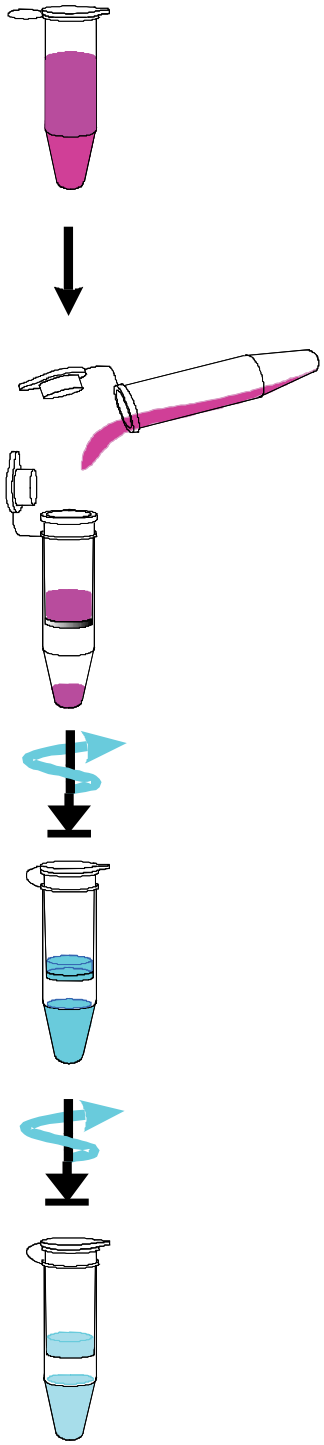
Host strains
Most *E. coli* strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid has an effect on the quality of the purified DNA. Host strains such as DH1, DH5α, and C600 give high-quality DNA. The slower growing strain XL1-Blue also yields DNA of very high-quality which works extremely well for sequencing. Strain HB101 and its derivatives, such as TG1 and the JM series, produce large amounts of carbohydrates, which are released during lysis and can inhibit enzyme activities if not completely removed. In addition, these strains have high levels of endonuclease activity which can reduce DNA quality. The methylation and growth characteristics of the strain should also be taken into account when selecting a host strain.
XL1-Blue and DH5α are highly recommended for reproducible and reliable results.

Inoculation
Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures may lead to uneven plasmid yield or loss of the plasmid. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid.
The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent so that single colonies can be isolated. This procedure should then be repeated to ensure that a single colony of an antibiotic resistant clone can be picked. A single colony should be inoculated into 1–5 ml of media containing the appropriate selective agent, and grown with vigorous shaking for 12–16 hours. Growth for more than 16 hours is not recommended since bacterial culture overgrows and plasmid yields may be reduced.

Antibiotics
Antibiotic selection should be applied at all stages of growth. Many plasmids in use today do not contain the *par* locus which ensures that the plasmids segregate equally during cell division. Daughter cells that do not receive plasmids will replicate much faster than plasmid-containing cells in the absence of selective pressure, and can quickly take over the culture.
The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by β-lactamase which is encoded by the Plasmid linked *bla* gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where “satellite colonies” appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. Ampicillin is also very sensitive to temperature, in solution it should be stored frozen in single-use aliquots.

Culture media
Luria-Bertani (LB) broth is the recommended culture medium for use with Invisorb® Spin Plasmid Kits, since richer broths such as TB (Terrific Broth) or 2x YT lead to extremely high cell densities, which can overload the purification system. It should be noted that cultures grown in TB may yield 2–5 times the number of cells compared to cultures grown in LB broth. If these media are used, recommended culture volumes must be reduced to match the capacity of the Spin Filter membrane. If excess culture volume is used, alkaline lysis will be inefficient, the Spin Filter membrane will be overloaded, and the performance of the system will be unsatisfactory. Furthermore, the excessive viscosity of the lysate will require vigorous mixing, which may result in shearing of bacterial genomic DNA and contamination of the plasmid DNA. Care must also be taken if strains are used which grow unusually fast or to very high cell densities. In such cases, doubling the volumes of Solution A, B, and C may be beneficial. It is best to calculate culture cell density and adjust the volume accordingly. Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are in common use. Although different LB broths produce similar cell densities after overnight culture, plasmid yields may vary significantly.

Scheme of Invisorb® Spin Plasmid Mini *Two*



Please read protocols prior the start of the preparation !

Transfer 0.5 ml up to 2 ml of the overnight *E. coli* culture into a 1.5 ml or 2.0 ml microcentrifuge tube

Centrifuge for 1 min at maximum speed
(11.000 – 16.000 x g) (11.000 – 13.000 rpm)
Remove the supernatant completely
Resuspend the bacterial pellet in 250 µl **Solution A**

Add 250 µl **Solution B**, mix gently, but thoroughly (4-6 times)

Add 250 µl **Solution C,** mix gently by inverting the tube
Centrifuge for 5 min at full speed (12.000 – 16.000 x g)
(11.000 – 13.000 rpm)

Transfer the clarified supernatant onto the Spin Filter
Incubate for 1 min at RT
Centrifuge for 1 min at 11.000 x g (11.000 rpm)

Discard the filtrate
Add 750 µl **Wash Solution**
Centrifuge for 1 min at 11.000 x g (11.000 rpm)

Discard the filtrate

Centrifuge for 3 min at full speed (12.000 – 16.000 x g)
(11.000 – 13.000 rpm)

Place the Spin Filter into a new 1.5 ml Receiver Tube
Add 50 - 100 µl **Elution Solution**

Incubate for 1 min at room temperature
Centrifuge at 11.000 x g (11.000 rpm) for 1 min

Important notes

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 5). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps should be carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they become contaminated.
- Do not mix kit components with components from other kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.

Preparing reagents and buffers

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). (See at our webpage: www.stratec.com)

1. Label the needed amount of 2.0 ml receiver tubes (per sample: 2 receiver tubes).
2. Place spin filters into labeled 2.0 ml receiver tubes.
3. Label the needed amount of 1.5 ml receiver tubes (per sample: 1 receiver tube).

10 DNA extractions:
Wash Solution is ready to use!
50 DNA-extractions:
Add 48 ml 96-100% ethanol to the bottle Wash Solution
250 DNA-extractions:
Add 160 ml 96-100% ethanol to the bottle Wash Solution
500 DNA-extractions:
Add 160 ml 96-100% ethanol to each bottle Wash Solution

Important note

Incomplete removal of the bacteria culture medium will affect lysis and dilute the lysate.

Growth of bacterial cultures

Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–5 ml LB medium containing the appropriate selective antibiotic. Incubate for 12–16 h at 37°C with vigorous shaking.
Growth for more than 16 h is not recommended since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.

Harvest the bacterial cells by centrifugation at minimum 6.000 x g (8.000 rpm) in a table-top microcentrifuge for 3 min at room temperature. The bacterial cells can also be harvested in 15 ml centrifuge tubes at 5.400 x g (7.000 rpm) for 10 min at 4°C. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

Instructions

The following notes are valid for all protocols:

Note: *The centrifugation steps were made with the **Centrifuge 5415 D from Eppendorf.** The indicated **rpm amounts** refer to this centrifuge.*

Protocol 1: Preparation of pDNA from 0.5 –2.0 ml bacteria cultures

Please read the instructions carefully and conduct the prepared procedure !

---

1. Transfer from 0.5 ml up to 2 ml of the overnight *E. coli* culture into a 1.5 ml or 2.0 ml microcentrifuge tube. For larger volumes of starting material please contact our technical support.

2. Centrifuge for 1 min at maximum speed (12.000 – 16.000 g) (11.000 – 13.000 rpm) to pellet the cells; remove the supernatant as completely as possible.

3. Resuspend the cell pellet in 250 µl **Solution A** completely by vortexing or by pipetting up and down. For multiple processing of samples we recommend a vortexer.

Note*: No cell pellet or clumps should be visible!*

4. Add 250 µl **Solution B**, close the tube and mix carefully by inverting the tube 5 times. Do not perform the lysis step more than 5 min!

Important: *Do not vortex the tube to mix the suspension! This step is critical for the separation of bacterial chromosomal DNA from plasmid DNA. Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. This sheared chromosomal DNA is not precipitated by NaOH/SDS and contaminates the plasmid DNA.*

5. Add 250 µl **Solution C** and mix gently, but thoroughly, by shaking the tube 4-6 times. Centrifuge for 5 min at full speed (12.000 – 16.000 g) (11.000 – 13.000 rpm). Do not vortex!

6. During centrifugation time place needed amount of Spin Filters into the 2.0 ml Receiver Tubes.

7. Transfer (decant!) the clarified supernatant into the Spin Filter. Incubate for 1 min on to the Spin Filter. Centrifuge for 1 min at 11.000 x g (11.000 rpm).

8. Discard the filtrate. Add 750 µl **Wash Solution**. Centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate.

9. Centrifuge for 3 min at full speed (12.000 – 16.000 x g) (11.000 – 13.000 rpm) to complete removing the residual ethanol.

10. Place the Spin Filter into a new 1.5 ml Receiver Tube and add 50 - 100 µl **Elution Solution** directly onto the center of the Spin Filter surface. Incubate for 1 min at room temperature. Finally centrifuge at 11.000 x g (11.000 rpm) for 1 min to elute the plasmid DNA

Note: *To increase the final DNA yield we recommend using a higher volume of Elution Solution. A longer incubation time with Elution Buffer (up to 10 min) also leads to a slightly higher final yield. In order to increase the DNA concentration we recommend eluting in a smaller volume than 50 µl (minimal 30 µl), but this will lead to a reduction of yield.*

Attention: *For in vitro transcription application, please elute the plasmid DNA with ddH_2O.*

Note: *The Elution Solution contains EDTA*

Protocol 2: Purification of low-copy plasmids & cosmids from up to 10 ml bacteria cultures

Please read the instructions carefully and conduct the prepared procedure.

---

1. Transfer 1 - 10 ml of the overnight *E. coli* cultures grown in LB medium into a 15 ml Falcon Tube and spin down the bacterial cells.

Follow the protocol 1, but:

Note: *When using **10 ml culture** volume, it is recommended to **double the volumes** of Solution A, B, C.*

When plasmids or cosmids are bigger than 10 kb, prewarm **Elution Solution** (or water) to 70°C prior eluting DNA from the Spin Filter membrane.

A 10 ml overnight LB culture typically yields 5–10 µg DNA.

***Attention**:* *this is an additional application and the needed buffer volumes are not calculated in the provided buffers. Please order additional buffer, if you would like to perform this application in larger quantities (see Ordering information, page 19)*

Protocol 3: Purification of plasmid DNA from 2 ml gram positive bacteria cultures

Please read the instructions carefully and conduct the prepared procedure.

---

1. Transfer 0.5 ml up to 2 ml of the overnight culture into a 1.5 ml or 2.0 ml microcentrifuge tube.

2. Centrifuge for 1 min at maximum speed (12.000 – 16.000 g) (11.000 – 13.000 rpm) to pellet the cells; remove the supernatant as completely as possible.

3. Resuspend the cell pellet in 250 µl **Solution A** completely by vortexing or by pipetting up and down. Add 10 µl of lysozyme (10mg/ ml or according the producers instructions). Mix the suspension. For multiple processing of samples we recommend a vortexer.

4. Incubate for 10 min at 37°C

Follow protocol 1 starting at step 4

Protocol 4: Preparation of pDNA from 0.5 –2.0 ml bacteria cultures using the Invisorb® 96 Vacuum Manifold

Please read the instructions carefully and conduct the prepared procedure.

---

1. Transfer 0.5 ml up to 2 ml of the overnight *E. coli* culture into a 1.5 ml or 2.0 ml microcentrifuge tube. For larger volumes of starting material please contact our technical support.

2. Centrifuge for 1 min at maximum speed (12.000 – 16.000 x g) (11.000 – 13.000 rpm) to pellet the cells; remove the supernatant as completely as possible.

3. Resuspend the cell pellet in 250 µl **Solution A** completely by vortexing or by pipetting up and down. For multiple processing of samples we recommend a vortexer.

Note: *No cell pellet or clumps should be visible !*

4. Add 250 µl **Solution B**, close the tube and mix carefully by inverting the tube 5 times. Do not perform the lysis step more than 5 min!

Important: *Do not vortex the tube to mix the suspension! This step is critical for the separation of bacterial chromosomal DNA from plasmid DNA. Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. This sheared chromosomal DNA is not precipitated by NaOH/SDS and contaminates the plasmid DNA*

5. Add 250 µl **Solution C** and mix gently, but thoroughly, by shaking the tube 4-6 times. Centrifuge for 5 min at full speed (12.000 – 16.000 x g) (11.000 – 13.000 rpm). Do not vortex!

6. During centrifugation time place the waste tray in the base of the **Invisorb® 96 Vacuum Manifold** (see page 17) and place the **Adapter (20)** to the top of the base of the **Invisorb® 96 Vacuum Manifold**. Place needed amount of Spin Filters into the adapter positions of the **Invisorb® 96 Vacuum Manifold** and seal the non used positions of the **Invisorb® 96 Vacuum Manifold** with Sealing Foils (not provided).

7. Transfer (decant!) the clarified supernatant into the Spin Filter. Incubate for 1 min onto the Spin Filter at RT. Apply vacuum for 1 min at least 200 mbar or 150 mm Hg.

8. Add 750 µl **Wash Solution**. Apply vacuum for 1 min at least 200 mbar or 150 mm Hg.

9. Apply vacuum for 5 min at least 200 mbar or 150 mm Hg for complete removal of residual ethanol.

10. Place the Spin Filter into a new 1.5 ml Receiver Tube and add 50 - 100 µl **Elution Solution** directly onto the center of the Spin Filter surface. Incubate for 1 min at room temperature. Finally centrifuge at 8.000 x g (9.500 rpm) for 1 min to elute the plasmid DNA.

Note: *To increase the final DNA yield we recommend using a higher volume of Elution Solution. A longer incubation time with Elution Buffer (up to 10 min) leads also to a slightly higher final yield. In order to increase the DNA concentration we recommend to elute in a smaller volume than 50 µl (minimal 30 µl) but this will lead to a reduction of yield.*

Attention: *For in vitro transcription application, please elute the plasmid DNA with ddH_2O.*

Troubleshooting

Problem	Cause	Comments and suggestions
low yield of plasmid DNA	incorrect Wash Solution	prepare the **Wash Solution** exactly as described in the manual, don't forget the ethanol. Storage of Wash Solution with firmly fixed cap
	poor elution of pDNA	add the **Elution Solution** directly on the center of the Spin Filter
	conditions for bacterial cultures are not optimal	changing of the conditions (media, growing time and other)
	elution Solution incorrect	DNA is eluted only using low-salt buffer (e.g. **Elution Solution** or water). Elution efficiency depends on pH, maximum efficiency is achieved between pH 7.0 – 8.0. When using water for elution, make sure that the pH is within this range
	too much starting material	use only the recommended amount of bacterial suspension
contamination of the plasmid DNA with chromosomal DNA	the sample was mixed too vigorously during step 4 or step 5	follow exactly the protocol. Do not vortex at these steps. Mix the samples only by inverting the tubes carefully
	bacteria overgrown	reduce time for growing the bacterial culture
	lysis too long	lysis must not exceed 5 minutes
problems because of poor cleavage by restriction endonucleases or for other applications	contamination of the final plasmid DNA with salt components	wash the plasmid DNA bound on the Spin Filters as described
	contamination of the final DNA with ethanol	keep the given centrifugation time, (step 9) extend it if necessary (test the smell)
RNA contamination	RNAse digestion insufficient	check culture volume against recommended volumes, and reduce if necessary add more **RNase A** recover DNA by precipitating the eluate, digesting with **RNase A** and purifying on a new spin column
additional band below the super coiled plasmid DNA band	denaturated super coiled plasmid DNA	incorrect incubation in **Solution B** increased incubation time with **Solution B** can cause denaturation of super coiled plasmid DNA

Appendix

General notes on handling pDNA

Nature of DNA

The length and delicate physical nature of DNA require careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of DNA is necessary to ensure it will function well in various downstream applications. Damaged DNA could perform poorly in applications such as Southern blotting and long-template PCR.

Handling fresh and stored material before the extraction of pDNA

For the isolation of plasmid DNA from bacteria, use either fresh cultures or pellets that have been frozen and stored at -20°C.

Storage of pDNA

Store pDNA and other small circular DNAs at +2 to +8°C. Storing pDNA at -15 to -25°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Examples of Plasmids, Media, Antibiotics and Bacterial strains

Common Plasmid Vectors

Plasmid Vector and Derivatives	Copy Number	Origin of Replication	High Copy
pBR322	15 – 20	PMB1	
pUC	500 – 700	PMB1 (mut)	+
pACYC	10 –12	P15A	
pSC101	5	PSC101	
pGEM	300 – 400	PMB1 (mut)	+
pbluescript	300 – 500	ColE1	+
pTOPO	n.d.	PMB1 (mut)	+

Common Media for bacterial culture (1L)

H medium
10 g tryptone
8 g NaCl

Lambda broth
10 g tryptone
2.5 g NaCl

LB medium
10 g tryptone
5 g yeast extract
10g NaCl

SOC Medium
20 g Bacto tryptone
5 g yeast extract
100 mM Sodium chloride
2.5 mM Pottasium chloride
10 mM Magnesium chloride
20 mM Magnesium sulfate
20 mM Glucose

TB (terrific broth)
12 g Bacto tryptone
24 g Ba to yeast extract
4 ml glycerol
Add H_2O to 900 ml and autoclave, then add to above sterile solution 100 ml of a sterile solution of 0.17 M KH_2PO_4 and 0.72 M K_2HPO_4

Tryptone broth
10 g tryptone
5 g NaCl

2YT medium
16 g tryptone
10 g yeast extract
5 g NaCl

Common Antibiotics

Antibiotic	Stock Solution	Dissolved in	Working Concentration
Ampicillin	50 mg / ml	H_2O	20 – 60 µg / ml
Carbenicillin	50 mg / ml	H_2O	20 – 60 µg / ml
Chloramphenicol	34 mg / ml	H_2O	25 -175 µg / ml
Kanamycin	10 mg / ml	H_2O	10 – 50 µg / ml
Streptomycin	10 mg / ml	H_2O	10 – 50 µg / ml
Tetracycline	5 mg / ml	Ethanol	10 – 50 µg / ml

For further information see:
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Chapter 1 and Appendix A

Ordering information

Product	Package Size	Catalogue No.
Invisorb® Spin Plasmid Mini *Two*	10 preparations	1010140900
Invisorb® Spin Plasmid Mini *Two*	50 preparations	1010140200
Invisorb® Spin Plasmid Mini *Two*	250 preparations	1010140300
Invisorb® Spin Plasmid Mini *Two*	500 preparations	1010140400
Solution A	15 ml	1010146000
Solution B	15 ml	1010146100
Solution C	15 ml	1010146200
Wash Solution	18 ml	1010143700
Elution Solution	15 ml	1010144000
Invisorb® Plasmid HTS 96 Kit/C	2 x 96 preparations	7010300200
Invisorb® Plasmid HTS 96 Kit/C	4 x 96 preparations	7010300300
Invisorb® Plasmid HTS 96 Kit/C	24 x 96 preparations	7010300400
InviMag® Plasmid Kit/ KF96	1 x 96 preparations	7410300100
InviMag® Plasmid Kit/ KF96	5 x 96 preparations	7410300200

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